The Lactose-Casein (Maillard) Browning System: Volatile Components

Aldo Ferretti and Vincent P. Flanagan

Over 40 new compounds were isolated and identified from a cold-finger molecular distillate obtained from a dichloromethane extract of a lactose-casein browning system, in connection with studies on off-flavor development in dairy products. These compounds consist of 15 C-5 to C-14 furanics, five pyrazines, three pyrroles, three lactones, 2-acetylpyridine, acrolein, cyclopentanone, 2-methyltetrahydrofuran-3-one, acetoin, acetophenone, propiophenone, benz-aldehyde, benzyl alcohol, phenol, N-methyl-2-pyrrolidinone, isopropylideneglycerol, acetol, and its acetate ester. Only six of them have been previously identified in milk or milk-related systems. Four compounds of lipid origin were also identified. Mass spectrometry and gas-liquid chromatography (two polar and two nonpolar columns) were the main tools for identification. Mass spectral data are given in detail.

This investigation is the continuation of work of Ferretti et al. (1970) on the nonenzymatic browning of lactose-casein mixtures. The study of this model system is ultimately aimed at establishing, or excluding, contribution of the Maillard reaction to off-flavor development in milk and its manufactured products. Many literature references about work done elsewhere up to mid-1969 on subjects directly related to the present study have been cited previously (Ferretti et al., 1970).

This paper deals with the isolation and identification of potentially flavor significant compounds formed in a lactose-casein system during browning. The starting material consisted of a cold-finger molecular distillate obtained from a dichloromethane extract of the browned mixture. In the present investigation the system lactose-casein, as well as the mixture of unknown constituents which was subjected to gas-liquid chromatographic (glc) separation, was prepared by a somewhat modified procedure. Identification was based solely on glc retention times and mass spectra (ms), normally without resorting to trapping of individual compounds. This allowed us to work with a substantially smaller quantity of whole extract than in our previous work. Final comparison of glc and ms data with those of reference substances was carried out in all cases but one. Synthetic work was necessary where authentic samples were not commercially, or otherwise, available to verify interpretation of mass spectra. None of the compounds presently reported were detected previously by us, because a less efficient procedure was used. The glc-ms combination made possible the identification of many compounds which are present in too small amounts to be trapped and analyzed by infrared and high resolution mass spectrometry according to the previous procedure. Acquired experience in ms made its extensive use possible.

Many of the identified products are expected to be organoleptically significant; their flavor evaluation and correlation with off-flavors of dairy products will be dealt with elsewhere.

EXPERIMENTAL

Chromatographic Methods. The following columns, all stainless steel, DMCS treated, and with a glass liner at the injection port, were used for gas-liquid chromatography. Column A: 10-ft × 0.25-in. (o.d.) packed with 15% Triton × 305 plus 0.15% Versamid 900, on 60/80 Chromosorb W AW/DMCS treated. Temperature programming was from

70° to 200° C at 4° per min from the air peak. Column B: 10-ft × 0.25-in. (o.d.) packed with 20% Silicone SE-30 on 60/80 Chromosorb W AW/DMCS treated. Temperature programming was from 75° to 200° at 2° per min from air. For columns A and B, carrier gas was helium, supplied at 60 psi head pressure, the injection port temperature 235° C, and detector temperature 235° C. The instrument was a 5750 Hewlett-Packard, equipped with a thermal conductivity detector.

Column C was a 24-ft × 0.125-in. 1% Carbowax 20M High-Efficiency packed column (Hi-Pak) by Hewlett-Packard. Column D: 8.25-ft × 0.125-in. (o.d.) packed with 10% Silicone SE-30 on 100/120 Chromosorb W AW/DMCS treated. Columns C and D were placed in the oven of an LKB-9000 gas chromatograph mass spectrometer and the effluent gas directly scanned. The carrier gas (He) was supplied with a head pressure of 40 psi. Temperature programming was varied, depending upon the particular fraction being analyzed; injection port temperature was 200° C.

Figure 1 shows a typical semi-preparative chromatogram (of the total mixture) obtained with column A; the dotted vertical lines indicate where the cuts were made. Minor fluctuations along the time axis were observed between runs, and the chromatogram profile was taken as the determining factor in cut making.

Spectrometric Methods. The mass spectra were determined with an LKB-9000 spectrometer coupled with a glc column. Unless otherwise indicated, the ionizing energy was 70 eV, source temperature 290° C, and separator temperature 230° C. The infrared spectra, calibrated with polystyrene, were determined in carbon tetrachloride solution with a Beckman IR-5A instrument employing a beam condenser and an ultramicro NaCl cell (0.025 mm thickness).

Preparation of Dichloromethane Extract. Twenty-four-hundred grams of Vitamin Free Casein (Nutritional Biochemicals Corp., Cleveland, Ohio) was defatted by a 48-hr Soxhlet extraction with rectified dichloromethane, and oven-dried at 40° C. The washed casein was then suspended in deionized water, the pH cautiously brought to 6.5 with 0.1 N sodium hydroxide under vigorous mechanical stirring, and 528 g of α-lactose (Fisher certified) was added. The ratio casein to lactose was the same as the one previously employed (Ferretti et al., 1970). After thorough stirring the mixture was freeze-dried in a Vacuum Shelf Dryer (New York Engineering Co., Yonkers, N.Y.) equipped with an Ingersoll-Rand steam injection system, operating at 100 lb steam pressure which afforded a vacuum of ca. 1 Torr. Shortly after the operating vacuum was reached, the emulsion solidified, and, from then

Dairy Products Laboratory, Eastern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, Washington, D.C. 20250
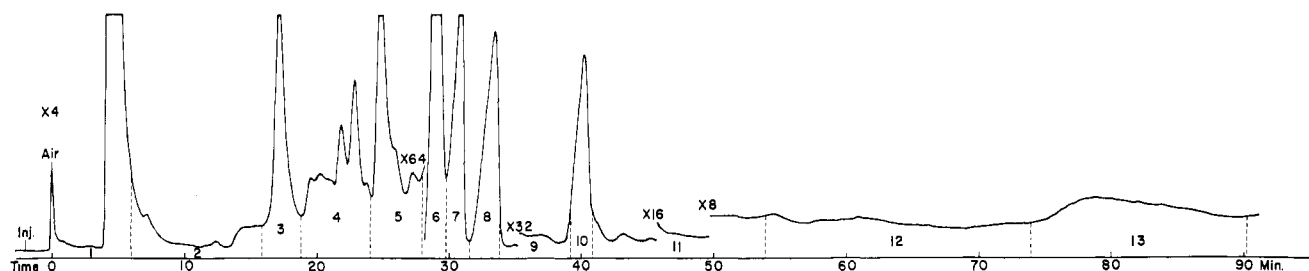
Figure 1. Gas-liquid chromatogram (time *vs.* instrument response) of a dichloromethane extract of a browned lactose-casein mixture. For conditions, see Experimental

on, enough heat was supplied to make the drying proceed at an acceptable rate without causing melting. Recovery of dried material, owing to the bulky nature of the operation, was not quantitative. The dry product, after grinding in a mortar, consisted of shiny amorphous flakes; yield was 2334 g; moisture content was found to be 4.75%. The lactose-casein powder was allowed to undergo browning in an atmosphere of 75% relative humidity in an Environ-Room (Lab-Line Instruments, Inc., Melrose Park, Ill.) at 75° C for 11 days. Then the powder, which had become light brown, was quickly ground in a mortar and immediately extracted in a Soxhlet with rectified dichloromethane for 30 hr. Careful evaporation of the solvent under a 33-cm helices-packed column, followed by brief pumping with an aspirator at room temperature, resulted in 14 ml of a brownish-orange, highly odorous, oily residue, which appeared to be contaminated with a small amount of butter oil. In addition, crystalline maltol readily precipitated on standing in a refrigerator. All of the butter oil and most of maltol were removed by a combination of centrifugings and acetone extractions. Purification resulted in 1 ml of butter oil, 1.4 g of pure maltol, and 11.1 ml of defatted oil. The latter was first subjected to cold-finger molecular distillation under high vacuum, then deacidified. De-acidification was accomplished by washing a CH_2Cl_2 solution of the whole oil with the minimum amount of a saturated sodium bicarbonate solution. Aqueous washings were saturated with salt and back-extracted with dichloromethane to recover any water soluble nonacid constituent. The resulting clear, pale yellow oil, 2.14 g, which will be referred to as the dichloromethane extract, was analyzed by glc and ms.

The above procedure for the preparation of the dichloromethane extract is similar to the one previously followed (Ferretti *et al.*, 1970), but major differences were introduced. The use of a Vacuum Shelf Dryer was dictated by the size of the sample to be dried. Defatting, by centrifuging and acetone extraction, was necessary because there was enough milk fat in the crude extract to seriously interfere with the direct glc-ms analysis. Acetic acid was also present in relatively large quantity, thus obscuring other compounds present in the same cut. It was also found that an excess of acetic acid causes peak broadening of the pyrazines. Therefore, it had to be removed. Washing with bicarbonate, however, did not effect total removal of some of the higher fatty acids (see below). Finally, prefractionation by cold-finger molecular distillation afforded a final mixture free from high boiling and nonvolatile materials.

Analysis of Dichloromethane Extract. The dichloromethane extract was subdivided into 14 cuts by repeated 40–50 μl injections on column A and trappings in capillary glass tubes cooled with dry ice. Figure 1 shows a typical chromatogram and approximate time limits within which each cut was made. The products of cuts no. 1, 2, and 3 were analyzed

with column C. Identification was made by mass spectra and retention times. The content of cuts 4 through 14 was analyzed with column D. However, cuts 6, 7, and 8 were found to consist largely of furfuryl alcohol, butyrolactone, and 2-butenoic acid γ-lactone, respectively, making the identification of the other compounds within these cuts rather problematic. Their necessary removal was done by using the high-phase column B: all compounds emerging from column B, except the three just mentioned, were trapped in capillary tubes and analyzed with column D like all other cuts from 4 through 14. In this work an unknown compound is considered to be identified when its gas-liquid chromatographic retention times (columns A and D) and mass spectrum have been shown to be identical with those of an authentic sample. The newly identified compounds are reported in Table I, in which they are listed according to the order of elution from the Triton column. The eight strongest mass spectral peaks (m/e) are reported; their intensities relative to that of the base peak (100%) are given in parentheses. Cut 14 was found to consist only of the previously identified 3-hydroxybutyrolactone.

Among the four possible isomeric C-3-alkyl substituted pyrazines, only the presence of trimethyl pyrazine was firmly established owing, primarily, to the outstanding features of its mass spectrum. Fast scanning mass spectrometric data, when column D was used, suggested that at least another alkyl pyrazine of molecular weight 122, namely a methyl ethyl pyrazine, must be present. The gas-liquid chromatogram of the C-3-alkyl pyrazines area on a 500-ft × 0.03-in. Silicone SF-96 capillary column clearly showed that four isomers, reasonably well resolved, were present. However, mass spectral confirmation for all of them could not be provided, and the identification of the methylethyl isomers remains tentative. An analogous situation presented itself in the case of the C-4-alkyl pyrazines, and, also this time, the singularity of the mass spectrum of tetramethyl pyrazine made its identification obvious. Fast scanning mass data of the eluate from column D indicated that only one isomeric C-5-alkyl pyrazine was present, namely trimethylethyl pyrazine. However, the presence of the 2,5-diethyl-3-methyl isomer could not be ruled out, since an authentic sample of this was not available for comparison. Several pyrazines were supplied by Dr. Victor Krampl of the Corporate Research Department of the Coca-Cola Company, Atlanta, Ga. The other reference materials, if not commercially available, were synthesized as described below. In all cases they were purified by glc before determination of their spectral properties.

In addition to the compounds reported in Table I, the following, of clear lipid origin, were also isolated and identified: decanoic acid, methyl decanoate, myristic acid, and methyl palmitate.

Synthesis of Authentic Materials. Cyclopentanone was

Table I. Data on Volatile Compounds Identified in a Lactose-Casein Browning System[a]

	Compound	Found Cut No.	Elution order in Column D	Mass Spectrum, m/e
1	Acrolein	1	1	27(100), 56M⁺(70), 28(60), 26(53), 55(49), 29(40), 25(8), 53(5)
2	Cyclopentanone	2	4	55(100), 84M⁺(39), 41(34), 56(29), 27(20), 42(15), 39(14), 26(8)
3	2-Methyltetrahydro-furan-3-one	3	5	43(100), 72(38), 29(24), 100M⁺(23), 44(23), 45(19), 27(16), 78(4)
4	3-Hydroxy-2-butanone	3	3	45(100), 43(62), 27(10), 88M⁺(9), 15(9), 29(7), 19(7), 42(5)
5	Acetol	3	2	43(100), 31(20), 74M⁺(12), 15(10), 29(8), 42(5), 27(3), 44(3)
6	Acetol acetate	4	7	43(100), 86(10), 116M⁺(6), 15(6), 73(5), 42(4), 44(3), 29(3)
7	Methylethylpyrazine	4	13	[b]
8	Trimethylpyrazine	4	14	42(100), 122M⁺(82), 39(21), 81(18), 40(13), 54(10), 27(8), 53(7)
9	2-Furaldehyde	4	6	96M⁺(100), 95(88), 39(60), 38(18), 29(16), 37(10), 40(6), 67(5)
10	2-Furfuryl formate	5	8	81(100), 126M⁺(43), 53(43), 80(28), 52(26), 27(18), 39(15), 29(13)
11	C-4-Alkylpyrazine	5	26	[b]
12	Tetramethylpyrazine	5	27	54(100), 136M⁺(96), 42(52), 53(19), 39(17), 27(14), 52(7), 51(6)
13	Benzaldehyde	5	11	105(100), 77(91), 106M⁺(66), 51(38), 50(18), 78(16), 73(9), 52(8)
14	Trimethylethyl- or 2,5-diethyl-3-methyl-pyrazine	5	33	149(100), 150M⁺(84), 39(30), 122(23), 53(23), 42(23), 54(21), 41(19)
15	2,2'-Bifuran	6	20	134M⁺(100), 78(58), 105(32), 51(31), 39(17), 77(14), 52(14), 50(11)
16	1-(2'-Furyl)-2-butanone	6[c]	21	57(100), 81(69), 29(63), 123(29), 138M⁺(24), 82(17), 95(4), 59(2)
17	2,2-Dimethyl-4-hydroxymethyl-1,3-dioxolane	6	10	43(100), 117(34), 101(23), 57(23), 59(18), 61(14), 42(11), 31(10)
18	2-Acetylpyridine	6	19	79(100), 78(85), 121M⁺(65), 43(44), 52(37), 51(36), 93(30), 50(13)
19	2-Furfuryl vinylacrylate	6	37	81(100), 53(24), 27(10), 178M⁺(9), 97(9), 82(8), 52(8), 133(6)
20	N-Methyl-2-formyl-pyrrole	6	15	109M⁺(100), 108(85), 53(45), 39(36), 80(33), 27(12), 38(9), 42(9)
21	2-Methylbenzofuran	6	29	131(100), 132M⁺(83), 77(15), 51(15), 103(12), 78(8), 63(7), 50(6)
22	1-(2'-Furyl)-3-butanone	7	24	81(100), 138M⁺(83), 43(72), 95(72), 53(20), 67(19), 39(15), 82(12)
23	N-Methyl-2-acetyl-pyrrole	7	25	108(100), 123M⁺(69), 53(34), 80(24), 39(24), 43(10), 109(7), 124(6)
24	Acetophenone	7	23	105(100), 77(71), 120M⁺(31), 51(23), 43(12), 50(9), 78(8), 106(7)
25	4-Methyl-2-butenoic acid γ-lactone	7	9	55(100), 43(63), 98M⁺(53), 83(52), 27(33), 54(26), 26(20), 69(15)
26	3-4-Dimethyl-2-butenoic acid γ-lactone	7	22	69(100), 41(57), 39(34), 97(21), 43(20), 40(20), 112M⁺(19), 68(16)
27	5-Methyl-2-propionyl-furan	8	30	109(off scale), 138M⁺(100), 53(67), 27(52), 110(31), 29(26), 43(22), 51(18)
28	N-Methyl-2-pyrrolid-inone	8	18	99M⁺(100), 44(86), 98(68), 42(64), 41(40), 43(20), 71(12), 39(12)
29	Propiophenone	8	31	105(100), 77(51), 51(19), 134M⁺(13), 106(8), 50(6), 27(4), 78(4)•
30	2-Methyl-5-(5'-methyl-2'-furfuryl)furan	8	35	176M⁺(100), 43(95), 161(58), 119(48), 133(33), 105(33), 91(21), 95(20)
31	Benzyl alcohol	9	17	79(100), 108M⁺(94), 107(72), 77(53), 51(24), 91(17), 78(12), 50(12)
32	Methyl 2-thiofuroate	9	32	95(100), 142M⁺(29), 39(15), 96(6), 38(5), 45(4), 67(4), 143(2)
33	2,3-Dimethyl-2-butenoic acid γ-lactone	9	28	55(100), 83(67), 112M⁺(48), 39(23), 27(18), 29(17), 53(13), 54(12)
34	Phenol	10	12	94M⁺(100), 66(23), 65(18), 39(16), 40(9), 95(7), 55(7), 38(5)
35	Difurfuryl ether	11	36	81(100), 82(48), 53(22), 97(10), 27(9), 39(9), 178M⁺(8), 41(6)
36	2-Formylpyrrole	11	16	95M⁺(100), 94(65), 66(47), 39(33), 38(11), 40(10), 41(8), 37(6)
37	5-(2'-Furfuryl)-2-furaldehyde	12[d]	39	176M⁺(100), 91(89), 147(51), 119(33), 65(22), 39(17), 53(14), 51(13)
38	5-Hydroxymethyl-2-furaldehyde[e]	13	34	97(100), 126M⁺(87), 69(18), 41(17), 124(9), 125(8), 109(5), 127(5)
39	5-(2'-Furfuryl)-2-furfuryl alcohol	13	38	178M⁺(100), 147(86), 91(63), 119(31), 81(25), 160(19), 53(17), 161(17)
40	2-(2'-Furfuryl)-5-(2''-furfuryl)-furan[e]	13	40	228M⁺(100), 147(46), 229(15), 91(11), 119(10), 148(5), 81(4), 189(4)

[a] The order of listing approximately follows the order of elution from column A; for conditions see Experimental. [b] For mass spectrum, see Experimental. [c] An authentic sample of this compound was not available; therefore its identification is tentative, and its exact eluting position in column A is not known. [d] This compound was found in cut no. 6; however, on the basis of its retention time, it belongs to cut no. 12; therefore it is probably an artifact. [e] Mass spectrum was determined at 20 eV.

prepared by dry distillation of barium adipate (Thorpe and Kon, 1964).

2-Methyl-tetrahydrofuran-3-one resulted from decarboxylative hydrolysis of 2-methyl-4-carbomethoxytetrahydrofuran-3-one (Gianturco et al., 1964).

Acetol acetate was obtained by (sulfuric) acid-catalyzed esterification of acetol.

Furfuryl formate was synthesized by reaction of furfuryl alcohol with acetic-formic mixed anhydride in the presence of sodium formate according to Edwards and Reeves (1942). The mixed anhydride was prepared from acetyl chloride and sodium formate in tetrahydrofuran (Schijf and Stevens, 1966).

2,2'-BIFURAN. This compound was obtained by radical coupling of furyl magnesium iodide in the presence of cobalt-

ous chloride as described by Atkinson *et al.* (1967), except that anhydrous ether was used in place of anhydrous tetrahydrofuran after a previous attempt with the latter solvent was unsuccessful. 2-Iodofuran was obtained by iodination of 2-chloromercurifuran according to the procedure of Gilman and Wright (1933).

2,2-Dimethyl-4-hydroxymethyl-1,3-dioxolane was prepared by acid catalyzed condensation of glycerol and acetone (Renoll and Newman, 1965).

2-Acetylpyridine resulted from dry distillation of a mixture of picolinic acid, calcium acetate, and (freshly calcined) calcium oxide (Engler and Rosumoff, 1891).

2-FURFURYL VINYLACRYLATE. Addition of a solution of vinylacrylic acid chloride in anhydrous benzene to furfuryl alcohol dissolved in benzene-pyridine afforded the ester which has not yet been reported in the literature. The intermediates vinylacrylic acid (Burton and Ingold, 1929) and its chloride (Alder *et al.*, 1949) were prepared according to procedures in the references cited. Ir: 2959(w), 1721(s), 1642 (m), 1603(m), 1499(m), 1435(w), 1416(w), 1366(m), 1302(s), 1263(s, broad), 1198(s), 1153(s), 1138(s), 1082(w), 1007(s, broad), 924(s), 886(m), and 867(m).

N-Methyl-2-formylpyrrole was prepared from *N*-methylpyrrole, POCl₃, and dimethylformamide according to a previously reported procedure (Silverstein *et al.*, 1955).

2-METHYLBENZOFURAN. Coumarilic acid chloride (Reichstein and Reichstein, 1930) in anhydrous xylene was subjected to the Rosenmund reduction with hydrogen in the presence of palladium on BaSO₄ poisoned with quinoline-sulfur poison (Rosenmund and Zetzsche, 1921). The resulting 2-formylbenzofuran was reduced with hydrazine hydrate-KOH to 2-methylbenzofuran as previously described (Bisagni *et al.*, 1955).

1-(2'-Furyl)-3-butanone was obtained from 2-furfuralacetone (Leuck and Cejka, 1964) by reduction with sodium amalgam and acetic acid (Harries and Kaiser, 1899).

N-METHYL-2-ACETYLPYRROLE. This compound was prepared by acylation of *N*-methylpyrrole with acetyl chloride in the presence of anhydrous magnesium bromide in ether (Herz, 1957).

4-Methyl-2-butenoic acid γ-lactone was obtained, along with the β-unsaturated isomer, by slow distillation of levulinic acid.

3,4-Dimethyl-2-butenoic acid γ-lactone was synthesized, starting from *o*-nitro-*p*-cresol according to the procedure of Pauly *et al.* (1914).

5-Methyl-2-propionylfuran was obtained by acylation of 2-methylfuran with propionic anhydride in the presence of boron trifluoride etherate.

2-Methyl-5-(5'-methyl-2'-furfuryl)furan was obtained in small yield, along with other products, when furfuryl alcohol was treated with 2-methylfuran in the presence of hydrochloric acid (Brown and Sawatzky, 1956).

METHYL 2-THIOFUROATE. Furoyl chloride, 6.5 g, (Hartman and Dickey, 1932) was dissolved in 25 ml of anhydrous pyridine, and methanethiol was bubbled through the ice-cooled solution for 45 min. The reaction mixture was poured into aqueous Na₂CO₃, the water solution salted out, and the product extracted with ether, dried and distilled. Yield was 2.4 g.

2,3-Dimethyl-2-butenoic acid γ-lactone was synthesized by bromination of ethyl 2,3-dimethyl-3-butenoate, followed by dehydrobromination and lactonization (Adams and Gianturco, 1957). The starting unsaturated ester was synthesized according to the procedure of Huston and Goerner (1946).

DIFURFURYL ETHER (35), 5-(2'-FURFURYL)-2-FURFURYL ALCOHOL (39) AND 2-(2'-FURFURYL)-5-(2''-FURFURYL)FURAN (40). When a 10% aqueous solution of furfuryl alcohol, brought to pH 2 with dilute sulfuric acid, was heated on a steam bath for 30 min, a heavy oil separated which was dissolved in ether, washed with sodium bicarbonate, and distilled under reduced pressure to remove polymeric materials. Gas-liquid chromatographic separation on a Silcone SE-30 column afforded, along with other products, the pure compounds 35, 39 and 40.

2-Formylpyrrole was obtained by formylation of pyrrole as described by Silverstein *et al.* (1963).

5-(2'-Furfuryl)-2-furaldehyde. This aldehyde, which so far has not been reported in the literature, also was prepared by the Vilsmeier-Haack formylation of 2,2'-difurylmethane; 1.5 grams of the latter in 3 ml of 1,2-dichloroethane was added slowly to a chilled mixture of 1 ml of POCl₃ and 0.85 ml of dimethylformamide. The mixture was then heated on a steam bath for 2 hr. After cooling, 10 g of CH₃COONa·3H₂O in 12 ml of water was added, the mixture extracted with ether, and the ether solution dried. After chromatography over neutral alumina (ether as solvent), the crude product (100 mg) was purified by glc. Ir: 3125(w), 2825(m), 1684 (s), 1587(m), 1513(s), 1401(m), 1311(m), 1279(m), 1200(m, broad), 1149(m), 1074(m), 1020(s), 1014(s), 980(m), 969(m), 938(m), and 883(m).

RESULTS AND DISCUSSION

The 40 new compounds identified during the present investigation are listed in Table I, which also shows the cut number where each compound has been found, the order of elution from a Silicone SE-30 column, and mass spectral data. Acetol, 5-hydroxymethyl-2-furaldehyde, 2-furaldehyde, phenol, acetophenone, benzaldehyde, and benzyl alcohol are, to our knowledge, the only compounds which have been previously identified in milk or milk-related systems. Benzaldehyde and phenol are typical products of caramelization of sugars (Hodge, 1967). However, benzaldehyde has recently been identified in the volatile nonacid extract of stored casein (Ramshaw and Dunstone, 1969), in stale sterile concentrated milk (Arnold *et al.*, 1966), in heated and raw milk (Scanlan *et al.*, 1968) and even in cream and butter oil fractions (Siek and Lindsay, 1968). Incidentally, Ramshaw and Dunstone (1969) have also found in stored casein 2-acetylfuran and 5-methyl-2-furaldehyde which were identified by us in the lactose-casein browning system (Ferretti *et al.*, 1970). Phenol too has been found in stored casein (Ramshaw and Dunstone, 1969) and other food systems.

Acrolein, 5-hydroxymethyl-2-furaldehyde, 2-furaldehyde, 3-hydroxy-2-butanone, and acetol are all well known products of carbohydrate fragmentation and dehydration during the nonenzymatic browning reaction (Heyns *et al.*, 1966; Hodge, 1967; Heyns and Klier, 1968; Shaw *et al.*, 1968). To them we can fairly safely add the following ones: acetol acetate, 2-furfuryl formate, 2,2'-bifuran, 1-(2'-furyl)-2-butanone, 1-(2'-furyl)-3-butanone, methyl 2-thiofuroate, 5-methyl-2-propionylfuran, and 2-furfuryl vinylacrylate.

Difurfuryl ether and 5-(2'-furfuryl)-2-furfuryl alcohol (39) might have arisen from (acid-catalyzed) intermolecular dehydration of furfuryl alcohol (Dunlop and Peters, 1953). The trifuryl compound 40 probably was formed by reaction of the alcohol 39 with furfuryl alcohol, with formation of an intermediate ether, followed by (thermally induced?) elimination of formaldehyde. 2-Methyl-5-(5'-methyl-2'-furfuryl)-furan (30) can be thought of as originating from condensation

of two molecules of 2-methylfuran and one molecule of form-aldehyde; the former was previously found in a similar lac-tose-casein browning system (Ferretti *et al.*, 1970), and the latter, although not yet identified, is quite possibly present in a Maillard system like the one under consideration. The point of whether the above reactions occurred in the browning mix-ture or during gas-liquid chromatography is debatable. In a study of thermal degradation of D-glucose, Heyns *et al.* (1966) suggested that furanic compounds are formed not from D-glucose itself but by fragmentation of polymers. This might indeed be the case for our compounds 15, 16, 19, 22, 27, and 32—even though our browning procedure is far from the pyrolytic conditions used by Heyns and coworkers—but almost certainly not for compounds 30, 35, 39, and 40. 5-(2′-Furfuryl)-2-furaldehyde (37) is probably an artifact be-cause it was found in cut No. 6 whereas, based on its retention time in column A, it should be found in cut No. 12.

2,2-Dimethyl-4-hydroxymethyl-1,3-dioxolane is, at least in part, of lipid origin, as it must have formed by acid catalyzed condensation of acetone and glycerol. 4-Methyl-2-butenoic acid γ-lactone is probably a product of the well-known hydro-lytic cleavage-rearrangement of furfuryl alcohol *via* the inter-mediacy of levulinic acid. However, levulinic acid itself was not identified.

Alkyl-substituted pyrazines of the type found in this investi-gation can be regarded as products of the Maillard browning. Some of them have been previously found in sugar-amino acid (Dawes and Edwards, 1966; Koehler *et al.*, 1969; Newell *et al.*, 1967; van Praag *et al.*, 1968), and sugar-ammonia (Hough *et al.*, 1952; Jezo and Luzak, 1966) browning mixtures, as well as in food systems other than milk, such as roasted pea-nuts (Mason *et al.*, 1969), coffee (Gianturco *et al.*, 1966; Goldman *et al.*, 1967; Stoffelsma *et al.*, 1968) and cocoa (Flament *et al.*, 1967; Marion *et al.*, 1967; Rizzi, 1967; van Praag *et al.*, 1968), where carbohydrates are heated in the presence of basic materials at some stage of processing.

None of the pyrrole derivatives identified in this work have so far been found in milk or milk related systems; all of them, however, have been found in either coffee (Gianturco *et al.*, 1966; Stoll *et al.*, 1967) or cocoa aroma (Marion *et al.*, 1967). In a recent paper, Kato and Fujimaki (1968) discussed a possible relationship between formation of 2-formylpyrrole and that of melanoidins in the nonenzymatic browning be-tween D-xylose and amino compounds.

Acetophenone, propiophenone, benzyl alcohol, 3,4-di-methyl-2-butenoic acid γ-lactone, N-methyl-2-pyrrolidinone, 2-methylbenzofuran, 2-acetylpyridine, 2,3-dimethyl-2-buten-oic acid γ-lactone, cyclopentanone, and 2-methyltetra-hydrofuran-3-one are not easily accountable on the basis of the known pathways of either the Maillard reaction or of the sugar caramelization. All of these have been previously found in nondairy food systems, with the only exception of acetophenone and benzyl alcohol. The former was also identified in heated (Scanlan *et al.*, 1968) and stale sterile con-centrated milk (Arnold *et al.*, 1966), as well as in stored casein (Ramshaw and Dunstone, 1969); the latter appeared in stored casein (Ramshaw and Dunstone, 1969). Additional research will be necessary to find out whether they are prod-ucts of a more general casein-lactose interaction or of other degradative processes.

Of all the products identified in our laboratory in heated lactose-casein mixtures, the following are present, by far, in the largest concentration: acetic acid, acetol, 2-acetylfuran, 5-methyl-2-furaldehyde, furfuryl acetate, furfuryl alcohol, butyrolactone, 2-butenoic acid γ-lactone, maltol, and β-

hydroxybutyrolactone. All except acetol were reported pre-viously (Ferretti *et al.*, 1970). The flavor threshold of these compounds in skim milk has not been determined.

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